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Alkaloids and a pimarane diterpenoid from *Strychnos vanprukii*

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Abstract

From the stem of *Strychnos vanprukii*, a gluco-indole alkaloid, 3,4-dehydropalicoside, and a pimarane diterpenoid, 7β-hydroxypimara-8,15-dien-14-one, were isolated together with four known alkaloids: palicoside, 3,4,5,6-tetradehydropalicoside, akagerine and 17-*O*-methylakagerine. The structures of these compounds were elucidated based on spectroscopic evidence.

Keywords: *Strychnos vanprukii*; Loganiaceae; Gluco-indole alkaloids; 3,4-Dehydropalicoside; Pimarane diterpenoid; 7β-Hydroxypimara-8,15-dien-14-one

1. Introduction

The genus *Strychnos* of the family Loganiaceae is a rich source of indole alkaloids, and comprises about 200 species, 14 of which can be found in Thailand. Plants in this genus have been used in folk medicine and in arrow and dart poisons in many parts of the world. *Strychnos vanprukii* Craib (Thai name: thao chang) is an Asian species growing in India, Indochina, Borneo, the Malay peninsula and northern Thailand. This plant is a large woody climber found in evergreen forests (Griffin and Parnell, 1997). Phytochemical and pharmacological information on *S. vanprukii* has not been previously available. The present study deals with the isolation and structural elucidation of palicoside (**1**), 3,4,5,6-tetradehydropalicoside (**2**) and a new vincosan-type gluco-indole alkaloid, 3,4-dehydropalicoside (**3**). Two known corynanthean-type indole alkaloids, akagerine (**4**) and 17-*O*-methylakagerine (**5**), were also isolated. In addition, the presence of a new pimarane diterpenoid, 7β-hydroxypimara-8,15-dien-14-one (**6**), in the hexane extract of the stem of *S. vanprukii* is reported.

2. Results and discussion

The alcoholic extract of the stem of *S. vanprukii* was partitioned into hexane, $CHCl_3$ and aqueous MeOH fractions. The hexane fraction afforded compound **6**, whereas the $CHCl_3$ extract contained akagerine (**4**) and its 17-*O*-methyl analog (**5**) (Rolfsen et al., 1978). The aqueous MeOH fraction yielded three β-carboline glucoalkaloids: palicoside (**1**) (Tits et al., 1996) and 3,4,5,6-tetradehydropalicoside (**2**) (Brandt et al., 1999), both previously found in the African *Strychnos mellodora*, and compound **3**.

An ES–MS–MS measurement of **3** gave a molecular formula of $C_{27}H_{33}N_2O_9$, which is one hydrogen less than that of palicoside (**1**). The ^{1}H-NMR spectrum shows four aromatic proton signals of the unsubstituted A ring of the indole system and the presence of a quaternary N_b-methyl group. Similar to **1** and **2**, the presence of a seco-iridoid moiety identical to secologanin was evident from several characteristic signals (Brandt et al., 1999). The vinyl group appeared as two doublets at δ 5.28 ($J = 10.8$ Hz, H-18$_{cis}$) and δ 5.22 ($J = 17.9$ Hz, H-18$_{trans}$) coupled to the more downfield H-19 (doublet of doublet of doublets at δ 5.92). An olefinic proton signal at δ 7.29 (*s*) and an acetal proton resonance at δ 5.68 (*d*, $J = 7.6$ Hz) correspond to H-17 and H-21, respectively. The anomeric proton of the glucose moiety, which appeared as a doublet ($J = 8.2$ Hz, H-1′) at δ

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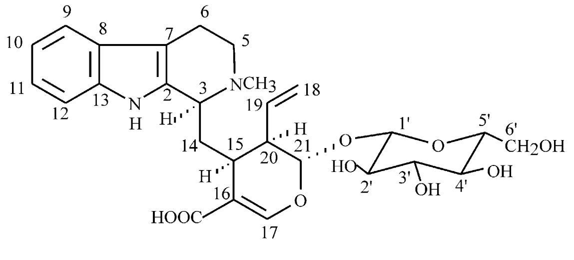
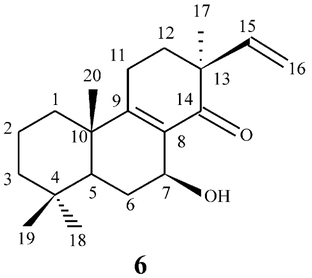
4.76, was consistent with the β-configuration of the sugar linkage.

The ^{13}C-NMR spectrum shows 27 carbons including 8 aromatic carbons, one carboxyl carbon (δ 174.4), two vinyl carbons (δ 135.7 and 120.3), one acetal carbon (δ 96.8), and those of one glucose unit including an anomeric carbon (δ 100.6). Carbons of the secologanin part (C-16–C-21 and C-1′–C-6′) show similar shifts as in **1** (Morita et al., 1989). The deshielded value for the quaternary N_b-methyl protons (δ 3.69) are consistent with those of melinonine F and normelinonine F (Brandt et al., 1999). In addition to a carboxyl carbon, another low field shifted carbon at δ 170.1 was observed. In the HMBC spectrum, long-range correlations between this carbon at δ 170.1 and three proton signals of H-5 (δ 4.07), H-15 (δ 3.45) and N-CH$_3$ (δ 3.69) were observed, indicating that the signal at δ 170.1 should be assigned to C-3 and that a double bond exists between C-3 and N-4. The structure of alkaloid **3** was thus elucidated as 3,4-dehydropalicoside. The signals of H-14 and C-14 were missing from the corresponding ^{1}H- and ^{13}C-NMR spectra measured in CD$_3$OD. This characteristic phenomenon can be explained by proton-deuterium exchange caused by an equilibrium between the imine and enamine forms (Kitajima et al., 2000). The stereochemistry of H-15, H-20 and H-21 in the seco-iridoid unit was determined by comparison of their chemical shifts and coupling constants with those of **2** and desoxy-cordifoline (Brandt et al., 1999). Their similarities indicated the configurations for H-15, H-20 and H-21 of **3** to be α, α and β, respectively. These are the configurations commonly deduced from the proposed biosynthetic pathway of these alkaloids.

Compound **6** was obtained as a viscous oil from the hexane extract. The ES–MS–MS gave a molecular ion at *m/z* 303, corresponding to the molecular formula $C_{20}H_{30}O_2$. The FT-IR spectrum shows absorption bands at 3442 (OH), 1655 (C=O) and 3082 cm^{-1} (vinylidene group). The UV absorption maximum at 242 nm is characteristic of an α,β-unsaturated carbonyl group. Based on the presence of this carbonyl, two double bonds and a total unsaturation number of 6, this compound should possess a tricyclic structure. The ^{1}H-NMR spectrum shows four methyl singlets at δ 0.89, 0.94, 1.06 and 1.12. Three sets of one-proton signals at δ 5.85 (*dd*, $J=17.6$, 10.8 Hz, H-15), 5.06 (*dd*, $J=10.8$, 0.9 Hz, H-16$_{cis}$) and 5.01 (*dd*, $J=17.6$, 0.9 Hz, H-16$_{trans}$), together with the carbon signals at δ 115.4 and 141.6 indicated a vinylidene group. The key HMBC correlation between both signals at δ 5.01 and 5.06 to the carbon signal at δ 48.7 (C-13) confirmed the attachment of the moiety to this carbon position. The NOESY spectrum (CDCl$_3$) of **6** exhibited cross peaks between the β-oriented Me-20 and the axial H-11β (δ 2.28, *ddd*, $J=10.9$, 7.9, 5.5 Hz), and also between Me-17 and both methylene protons of position 12. Analysis of the coupling constants of H-12α signal (δ 1.72, *ddd*, $J=13.4$, 7.9, 1.4 Hz) indicated that the proton should also be axial. The α-orientation of Me-17 in a pimarane structure would then support the NOEs from this group to both H-12α and H-12β. Detailed examination of the Dreiding and computer-generated conformational models of **6** (Fig. 2) confirmed the assignment of this diterpenoid as a pimarane. The ^3J coupling between the carbonyl carbon at δ 204.2 and protons of H-12 (δ 1.75), H-15 (δ 5.85) and H-17 can be observed, indicating the keto group to be at C-14. The long-range coupling between a single proton signal at δ 4.63 (*ddd*, $J=4.4$, 1.5, 1.4 Hz, H-7) with the ^{13}C signals at δ 45.7 (C-5) and 132.1 (C-8), between H-6 (δ 1.84) and C-10 (δ 41.2), and consistency of these chemical shifts with those of the diterpenoids possessing a similar partial structure (Evidente et al., 2002) indicates the location of a hydroxyl group at C-7 and suggests a fully substituted olefinic double bond at C-8 and C-9. The stereochemistry of



1
2 : Δ^3, Δ^5
3 : Δ^3



6

Fig. 1.

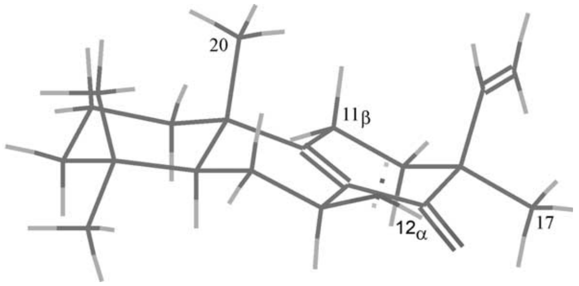


Fig. 2. Computer-generated model of **6** (23.59 kcal mol^{-1}).

compound **6** was established by NOE experiment. The doublet of doublet of doublets at δ 4.63 (H-7) showed a correlation with the signal of H-5 (δ 1.54) which is α-oriented, hence confirming the configuration of 7-OH as β. Therefore, compound **6** was elucidated as 7β-hydroxypimara-8,15-dien-14-one. Although monoterpenoids are readily found linked to the indole alkaloids of *Strychnos* species, to our knowledge, this is the first report of diterpenoids in the family Loganiaceae.

β-Carboline compounds are now extensively studied and found to exhibit various biological activities, for examples, hallucinogenic (Grella et al., 1998), neurotoxic (Riederer et al., 2002), cytotoxic (Beutler et al., 1993; Rashid et al., 2001), and antitumor (Ishida et al., 1999) activities. Palicoside (**1**) displayed antimycotic properties against *Candida albicans, C. glabrata* and *Aspergillus niger* in the presence of a specific glucosidase isolated from *Strychnos mellodora*. This gluco-alkaloid could be converted into akagerine (**4**), which is a monoterpenoid alkaloid with an N_a–C-17 linkage (Brandt et al., 2001). **4** displayed convulsant (Rolfsen et al., 1978) and antiprotozoal (Wright et al., 1991) activities in mice.

3. Experimental

3.1. General

UV spectra were obtained on a Milton Roy Spectronic 3000 Array Spectrometer. IR spectra were recorded on a Perkin Elmer FT-IR 1760x spectrophotometer. NMR spectra were recorded in CD_3OD using a Varian DPX-300 FT-NMR Spectrometer (300 MHz for ^{1}H-NMR and 75 MHz for ^{13}C-NMR) or a JEOL JNM-A 500 (alpha series) (500 MHz for ^{1}H-NMR and 125 MHz for ^{13}C-NMR). MS spectra were recorded on an Applied Biosystems Q-STAR (quadrupole-TOF) mass spectrometer. TLC was carried out using precoated Kieselgel 60 F_{254} plates (Merck). The spray reagents used for TLC were 10% H_2SO_4 in 95% EtOH and Dragendorff' s reagent. The optical rotations were measured with a Perkin Elmer 341 polarimeter.

3.2. Plant material

The stem of *Strychnos vanprukii* Craib was collected from Chaiyapum, Thailand, in 1998. The plant (voucher specimen No. RB9824) was identified by comparison with herbarium specimens kept at the Botany section, Technical Division, Royal Forest Department, Ministry of Agriculture and Co-operative, Thailand.

3.3. Extraction and Isolation

Dried and powdered stem of *S. vanprukii* (100 g) was extracted with EtOH to yield an EtOH extract (4.1 g) and an insoluble powder (3.1 g) precipitated while concentrating the extract. The insoluble powder was purified by washing with methanol to give compound **1** (2.8 g). The EtOH extract was dissolved in aqueous MeOH and partitioned with hexane followed by $CHCl_3$ to obtain hexane (257.8 mg), $CHCl_3$ (327.8 mg) and aqueous MeOH extracts (3.5 g). The MeOH extract was fractionated by silica gel CC using a gradient of $CHCl_3$–MeOH (4:1 to 3:2) to yield 7 major fractions. Fraction 6 (300 mg) was subjected to additional silica gel CC eluting with $CHCl_3$–MeOH (6:5) to yield **2** (8.5 mg) and **3** (15.8 mg). The $CHCl_3$ extract (327.8 mg) was subjected to silica gel CC with $CHCl_3$–MeOH (10:1) to yield **4** (5.3 mg) and **5** (2.8 mg). The hexane extract (258.7 mg) was separated on a silica gel column eluting with $CHCl_3$–hexane (3:1) to yield compound **6** (28.9 mg).

3.4. Palicoside (1)

For UV, MS, IR, ^{1}H-NMR and ^{13}C-NMR spectra, see lit. (Morita et al., 1989).

3.5. 3,4,5,6-Tetradehydropalicoside (2)

For UV, MS, IR, ^{1}H-NMR and ^{13}C-NMR spectra, see lit. (Brandt et al., 1999).

3.6. 3,4-Dehydropalicoside (3)

Amorphous powder; UV (MeOH) λ_{max} nm (log ϵ): 209 (4.00), 360 (3.70). IR (KBr) ν_{max} cm^{-1}: 3371 (OH), 1645 (C=O), 1390, 1077, 942. Positive ion mode ES–MS–MS *m/z*: 529 $[M]^+$, 367 $[529\text{-Glc}]^+$. Found: C, 61.4; H, 5.9. $C_{27}H_{33}N_2O_9$ requires: C, 61.3; H, 6.1%. $[\alpha]^{25}_{D}$ −27° (MeOH; *c* 0.175). ^{1}H-NMR (CD_3OD, 300 MHz): δ 2.74 (1H, *d*, J=7.6 Hz, H-20), 3.10–3.40 (4H, *m*, H-2′-4′), 3.29 (2H, *m*, H-6), 3.45 (1H, *m*, H-15), 3.50 (1H, *dd*, J=10.8, 6.2 Hz, H-6′a), 3.69 (3H, *s*, N-CH_3), 3.95 (1H, *d*, J=10.8 Hz, H-6′b), 4.07 (2H, *t*, J=8.3 Hz, H-5) 4.76 (1H, *d*, J=8.2 Hz, H-1′), 5.22 (1H, *d*, J=17.9 Hz, H-18_{trans}), 5.28 (1H, *d*, J=10.8 Hz, H-18_{cis}), 5.68 (1H, *d*, J=7.6 Hz, H-21), 5.92 (1H, *ddd*, J=18.1, 17.9, 10.8 Hz, H-19), 7.15 (1H, *t*, J=7.9, 7.3 Hz, H-10), 7.29 (1H, *s*, H-17), 7.40 (1H, *t*, J=8.2, 7.3 Hz, H-11), 7.55 (1H, *d*, J=8.2 Hz, H-12), 7.66 (1H, *d*, J=7.9 Hz, H-9). ^{13}C-NMR (CD_3OD, 75 MHz) δ: 20.1 (*t*, C-6), 36.9 (*d*, C-15), 43.3 (*q*, N-CH_3), 45.2 (*d*, C-20), 55.1 (*t*, C-5), 63.0 (*t*, C-6′), 71.7 (*d*, C-4′), 74.7 (*d*, C-2′), 78.0 (*d*, C-3′), 78.5 (*d*, C-5′), 96.8 (*d*, C-21), 100.6 (*d*, C-1′), 114.6 (*d*, C-12), 114.7 (*s*, C-16), 120.3 (*t*, C-18), 122.5 (*d*, C-9), 122.7 (*d*, C-10), 124.0 (*s*, C-7), 125.4 (*s*, C-8), 129.7 (*d*, C-11), 135.7 (*d*, C-19), 142.8 (*s*, C-13), 150.8 (*d*, C-17), 170.1 (*s*, C-3), 174.4 (*s*, C-22).

3.7. Akagerine (4)

For UV, MS, IR, ^{1}H-NMR and ^{13}C-NMR spectra, see lit. (Rolfsen et al., 1978).

Table 1
^{1}H and ^{13}C-NMR spectroscopic data of compound (**6**)

Position	δ_C	δ_H[b]	δ_H	HMBC (H/C correlations)[a]
1	36.3 *t*	1.86 *m*	1.85 *m*	C-10
		1.18 *m*	1.15 *m*	C-20
2	19.7 *t*	1.64 *tt* (13.7, 3.4)	1.70 *tt* (13.7, 3.4)	
		1.47 *m*	1.54 *m*	
3	42.4 *t*	1.42 *dddd* (13.4, 3.4, 3.4, 1.2)	1.46 *dddd* (13.4, 3.4, 3.4, 1.2)	
		1.22 *m*	1.23 *td* (13.4, 4.0)	C-2, C-4, C-18, C-19
4	34.0 *s*			
5	45.7 *d*	1.47 *dd* (13.1, 1.8)	1.54 *dd* (13.1, 1.6)	C-3, C-4, C-6, C-7, C-18, C-19, C-20
6	28.4 *t*	1.86 *ddd* (14.2, 1.8, 1.4)	1.84 *ddd* (13.7, 1.6, 1.5)	C-5, C-7, C-10
		1.59 *td* (14.2, 5.2)	1.63 *td* (13.7, 4.4)	
7	63.1 *d*	4.58 *dd* (5.2, 1.4)	4.63 *ddd* (4.4, 1.5, 1.4)	C-5, C-8
8	132.1 *s*			
9	169.8 *s*			
10	41.2 *s*			
11α	23.2 *t*	2.30 *ddd* (10.9, 4.6, 1.4)	2.37 *ddd* (8.1, 4.0, 1.4)	C-8, C-9, C-12, C-13
11β		2.28 *ddd* (10.9, 7.9, 5.5)		
12α	36.7 *t*	1.72 *ddd* (13.4, 7.9, 1.4)	1.75 *dt* (13.4, 8.1)	C-9, C-11, C-13, C-14, C-15
12β		1.91 *ddd* (13.4, 5.5, 4.6)	1.97 *dt* (13.4, 4.0)	
13	48.7 *s*			
14	204.2 *s*			
15	141.6 *d*	5.74 *dd* (17.5,10.8)	5.85 *dd* (17.6,10.8)	C-12, C-13, C-14, C-17
16_{cis}	115.4 *t*	5.06 *dd* (10.8, 0.9)	5.06 *dd* (10.8, 0.9)	C-13, C-15
16_{trans}		4.95 *dd* (17.5, 0.9)	5.01 *dd* (17.6, 0.9)	
17	24.3 *q*	1.16 *s*	1.12 *s*	C-12, C-13, C-14, C-15
18	33.3 *q*	0.93 *s*	0.94 *s*	C-3, C-4, C-5, C-19
19	22.2 *q*	0.85 *s*	0.89 *s*	C-3, C-4, C-5, C-18
20	18.3 *q*	1.01 *s*	1.06 *s*	C-1, C-5, C-9, C-10

[a] Correlation from H to the indicated carbons.
[b] ^{1}H-NMR spectra were recorded in $CDCl_3$.

3.8. *17-O-Methylakagerine (**5**)*

For UV, MS, IR, ^{1}H-NMR and ^{13}C-NMR spectra, see lit. (Rolfsen et al., 1978).

3.9. *7β-Hydroxypimara-8,15-dien-14-one (**6**)*

A viscous oil; UV (MeOH) λ_{max} nm (log ϵ): 242 (3.56). IR (KBr) ν_{max} cm^{-1}: 3442 (OH), 3082 (vinylidene group), 1655 (C=O). Positive ion mode ES–MS–MS m/z: 303 $[M+H]^+$. Found: C, 79.6; H, 9.8. $C_{27}H_{32}N_2O_9$ requires: C, 79.4; H, 10.0%. $[\alpha]_D^{25}$ +77° (MeOH; c 0.2). For ^{1}H-NMR (CD_3OD and $CDCl_3$) and ^{13}C-NMR (CD_3OD) spectral data, see Table 1.

Acknowledgements

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